FIG. 1

```
                   10            20                 30                   40                  50
Bet v 1 sense       5'- A A T T A T G A G A C T G A G A C C A C C T C T G T T A T C C C A G C A G C T C G -3'
Bet v 1 non-sense   3'- T T A A T A C T C T G A C T C T G G T G G A G A C A A T A G G G T C G T C G A G C -5'
sense primer        5'-                       T G A G A C C C C C T C T G T T A T C C C A G               -3'
non-sense primer    3'-       A T A C T C T G A C T C T G G G G G A G A C A                               -5'
```

FIG. 2

Oligonucleotide primers for site directed mutagenesis of Bet v 1 (No. 2801).

all	sense	1: 183Bv,	15-mer 5'-	G T T G C C A A C G A T C A G
1	sense	2: 184Bv,	23-mer 5'-	T G A G A C C C C C T C T G T T A T C C C A G
1	non-sense	3: 185Bv,	23-mer 5'-	A C A G A G G G G G T C T C A G T C T C A T A
2	sense	4: 186Bv,	31-mer 5'-	G A T A C C C T C T T T C C A C A G G T T G C A C C C C A A G
2	non-sense	5: 187Bv,	31-mer 5'-	A C C T G T G G A A A G A G G G T A T C G C C A T C A A G G A
3	sense	6: 188Bv,	23-mer 5'-	A A C A T T T C A G G A A A T G G A G G G C C
3	non-sense	7: 189Bv,	23-mer 5'-	T T T C C T G A A A T G T T T T C A A C A C T
4	sense	8: 190Bv,	23-mer 5'-	T T A A G A A C A T C A G C T T T C C C G A A
4	non-sense	9 191Bv,	23-mer 5'-	A G C T G A T G T T C T T A A T G G T T C C A
5	sense	10: 192Bv,	23-mer 5'-	G G A C C A T G C A A A C T T C A A A T A C A
5	non-sense	11: 193Bv,	23-mer 5'-	A G T T T G C A T G G T C C A C C T C A T C A
6	sense	12: 194Bv,	23-mer 5'-	T T T C C C T C A G G C C T C C C T T T C A A
6	non-sense	13: 195Bv,	23-mer 5'-	A G G C C T G A G G G A A A G C T G A T C T T
7	sense	14: 196Bv,	24-mer 5'-	T G A A G G A T C T G G A G G G C C T G G A A C
7	non-sense	15: 197Bv,	24-mer 5'-	C C C T C C A G A T C C T T C A A T G T T T T C
8	sense	16: 198Bv,	24-mer 5'-	G G C A A C T G G T G A T G G A G G A T C C A T
8	non-sense	17: 199Bv,	24-mer 5'-	C C A T C A C C A G T T G C C A C T A T C T T T
all	non-sense	18: 200Bv,	15-mer 5'-	C A T G C C A T C C G T A A G

FIG. 3

1 (A- C)

GGTGTGTTTAATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCGACTGTTCAAG 60

G V F N Y E T E T T-F S V I P A A R L F K 20

9 (A- G) 2 (A-C) 2 (A- C)

GCCTTTATCCTTGATGGCGATAACCTCTTTCCAAAGGTTGCACCCCAAGCCATTAGCAGT 120

A F I L D-G G D N-T L F P K-Q V A P Q A I S S 40

3(GA- TC) 7 (AA- TC) 4 (G- C) 6 (GA- T C)

GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAAGAAGATCAGCTTTCCCGAA 180

V E N I E-S G N-S G G P G T I K K-N I S F P E-S 60

5 (CA - TG)

GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCACACAAACTTCAAA 240

G L P F K Y V K D R V D E V D H T-A N F K 80

TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC 300

Y N Y S V I E G C P I G D T L E K I S N 100

10 (G AG -CAC) 8 (CCC - TGG)

GAGATAAAGATAGTGGCAACCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC 360

E I K I V A T P-G D G G S I L K I S N K Y 120

CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC 420

H T K G D H E V K A E Q V K A S K E M G 140

GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCTACAACTAA 480

E T L L R A V E S Y L L A H S D A Y N stop 159

FIG. 4

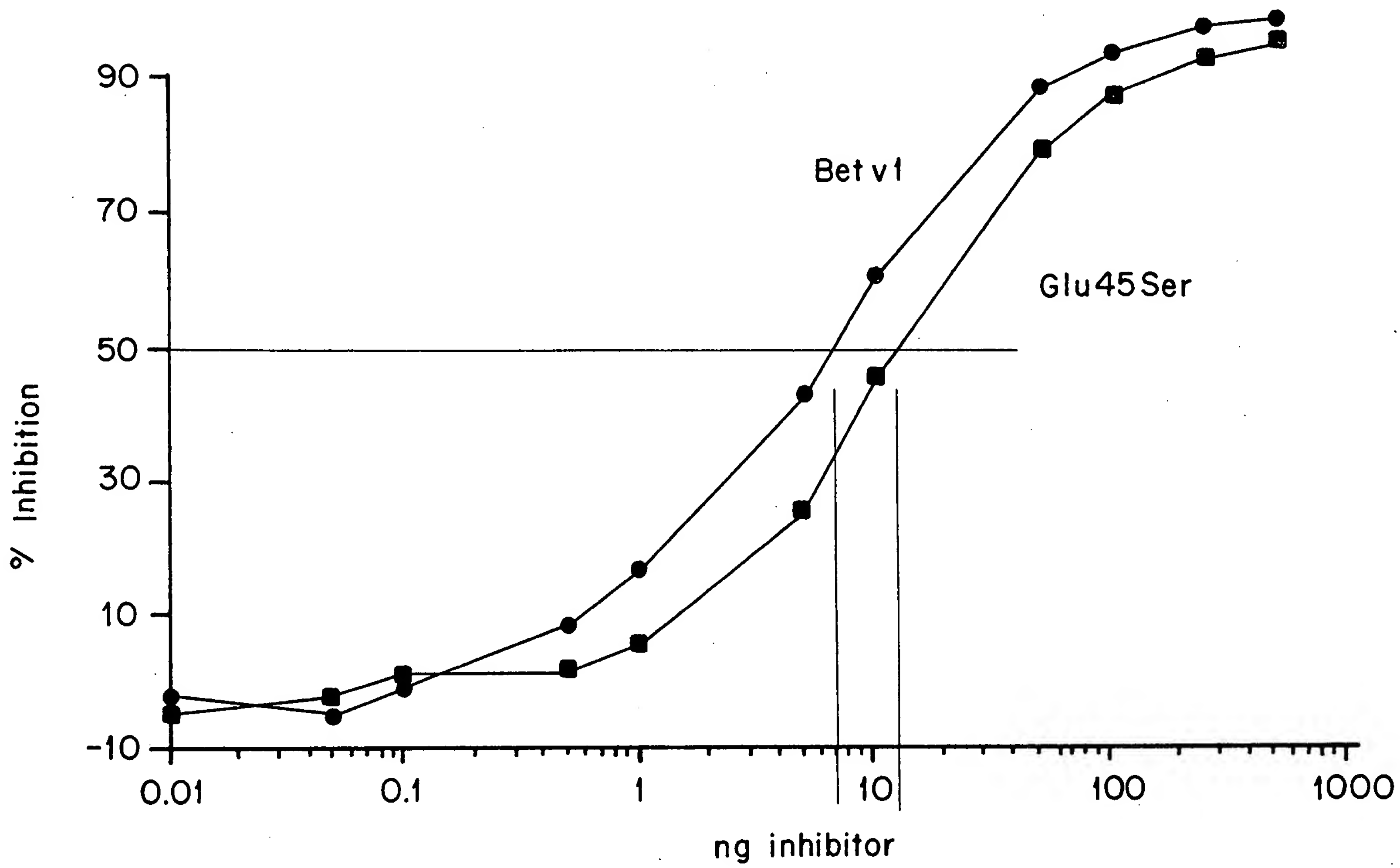

90
70
50
30
10
-10
% Inhibition
0.01
0.1
1
10
100
1000
ng inhibitor
Bet v1
Glu45Ser

FIG. 5

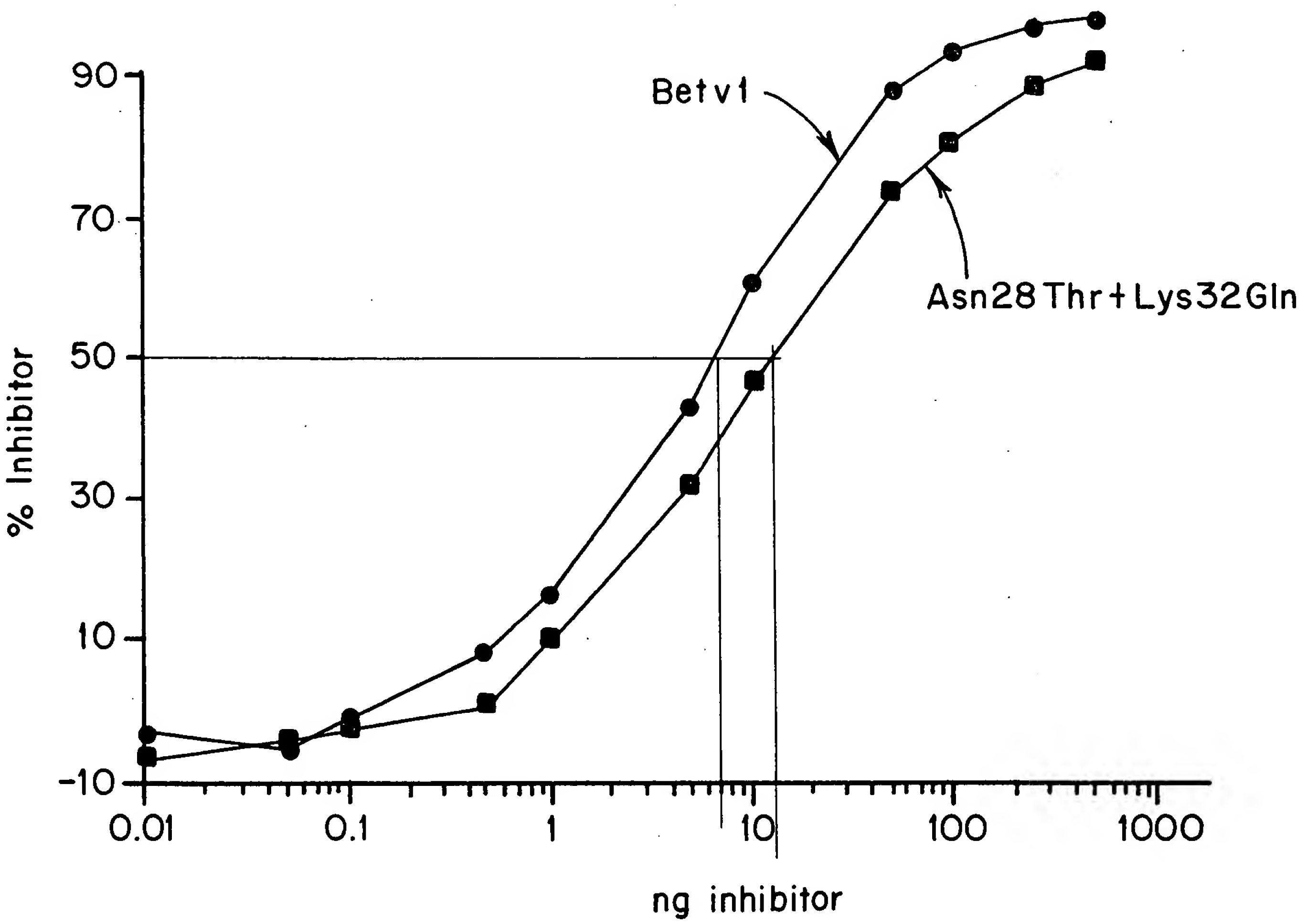

Betv1
Asn28Thr+Lys32Gln
% Inhibitor
90
70
50
30
10
-10
0.01
0.1
1
10
100
1000
ng inhibitor

FIG. 6

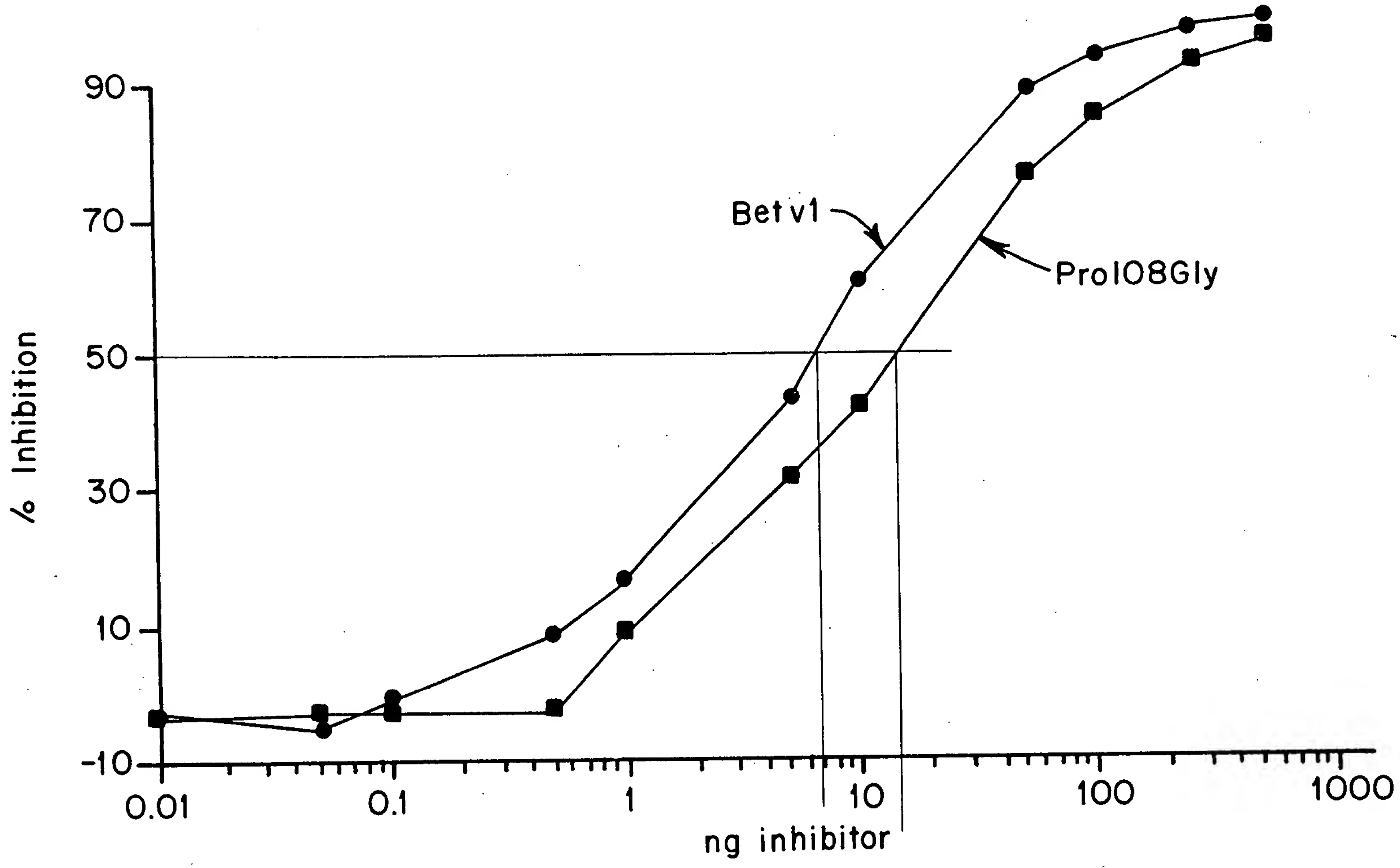
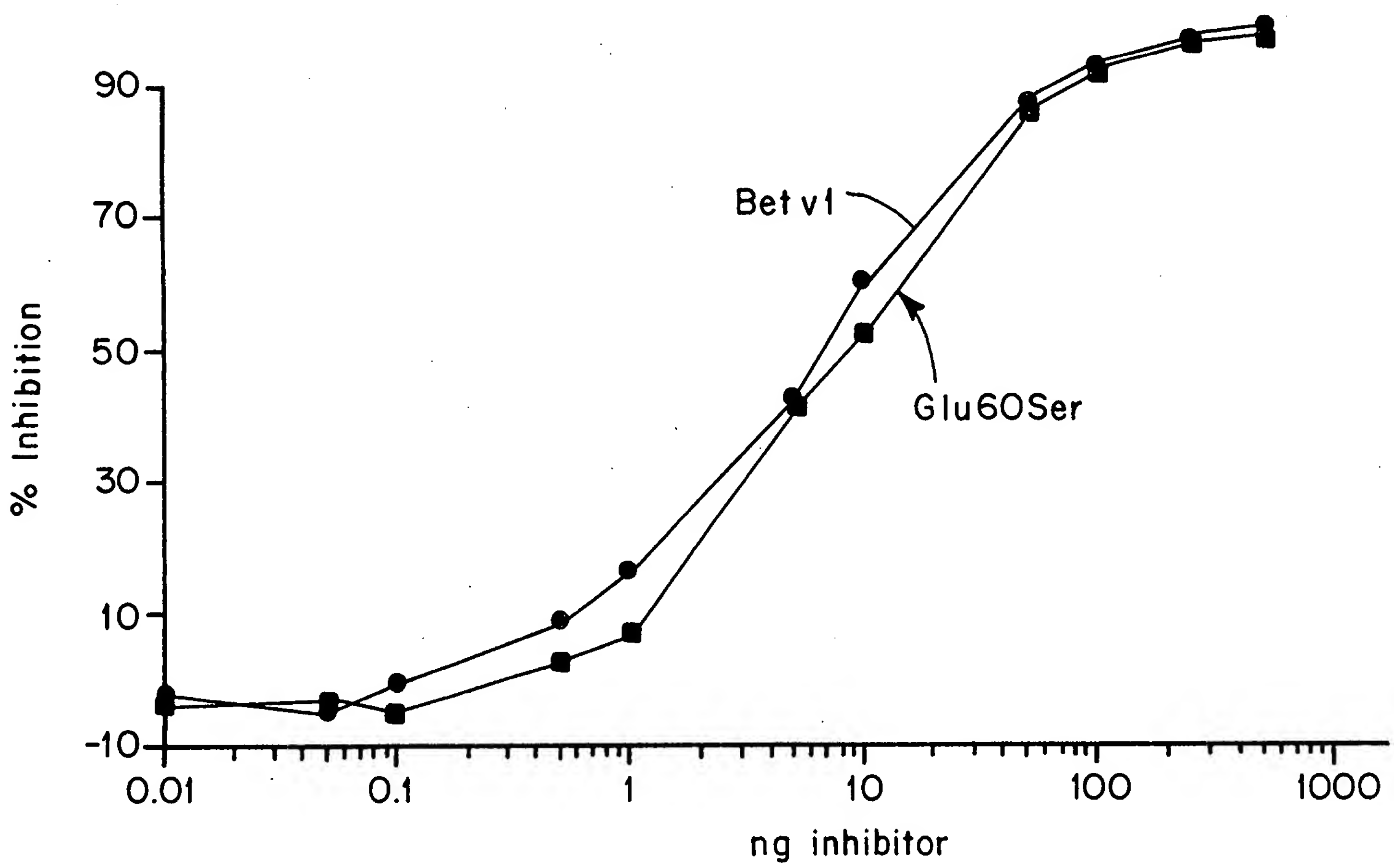

90
70
50
30
10
-10
% Inhibition
0.01
0.1
1
10
100
1000
ng inhibitor
Bet v1
Pro108Gly

FIG. 7


90
70
50
30
10
-10
% Inhibition
0.01
0.1
1
10
100
1000
ng inhibitor
Bet v1
Glu60Ser

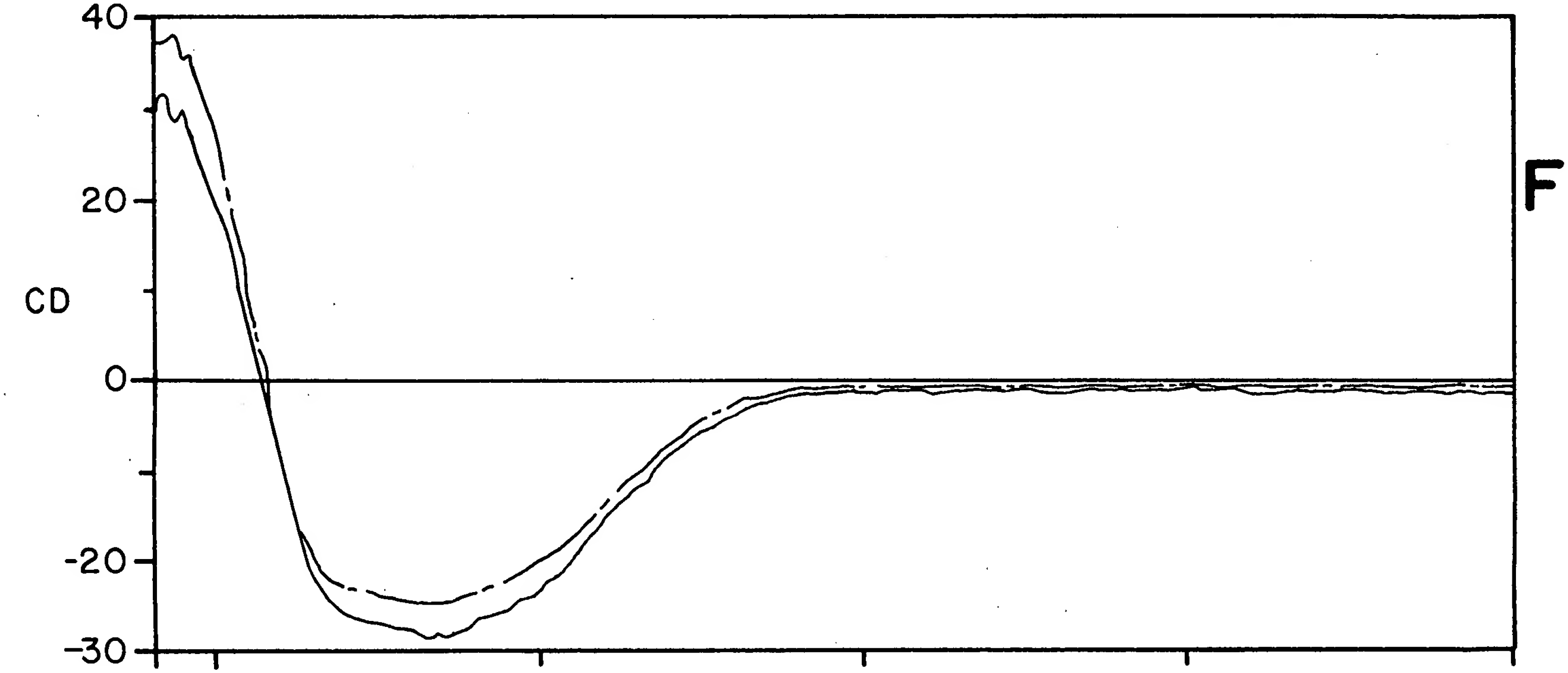

40
20
CD
0
-20
-30

FIG. 8A

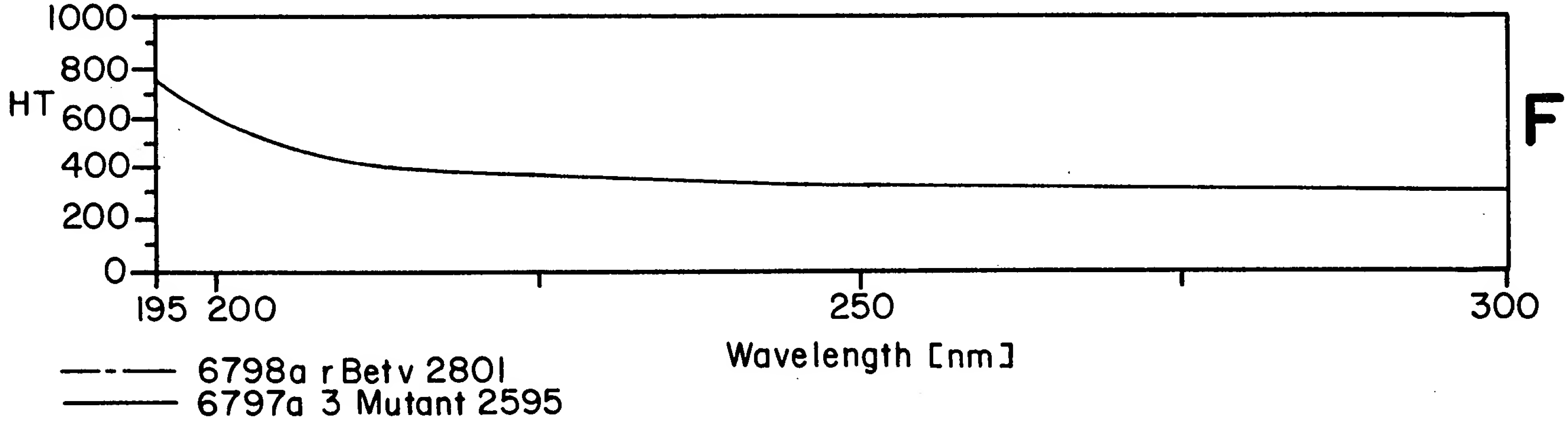

1000
800
HT
600
400
200
0
195
200
250
300
Wavelength [nm]
6798a r Bet v 2801
6797a 3 Mutant 2595

FIG. 8B

FIG. 9

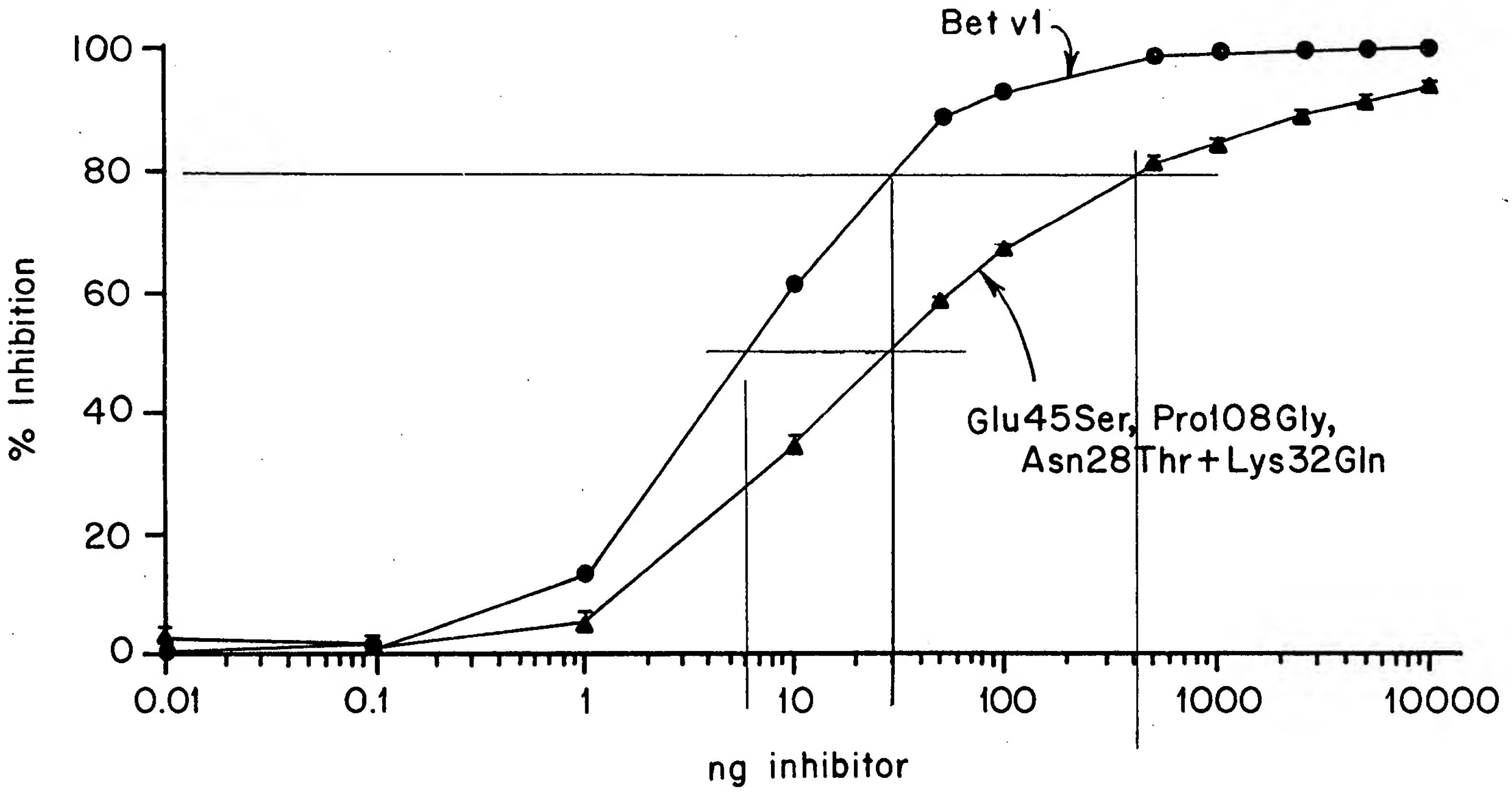

Bet v1
Glu45Ser, Pro108Gly,
Asn28Thr+Lys32Gln
% Inhibition
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
10000
ng inhibitor

FIG. 10A

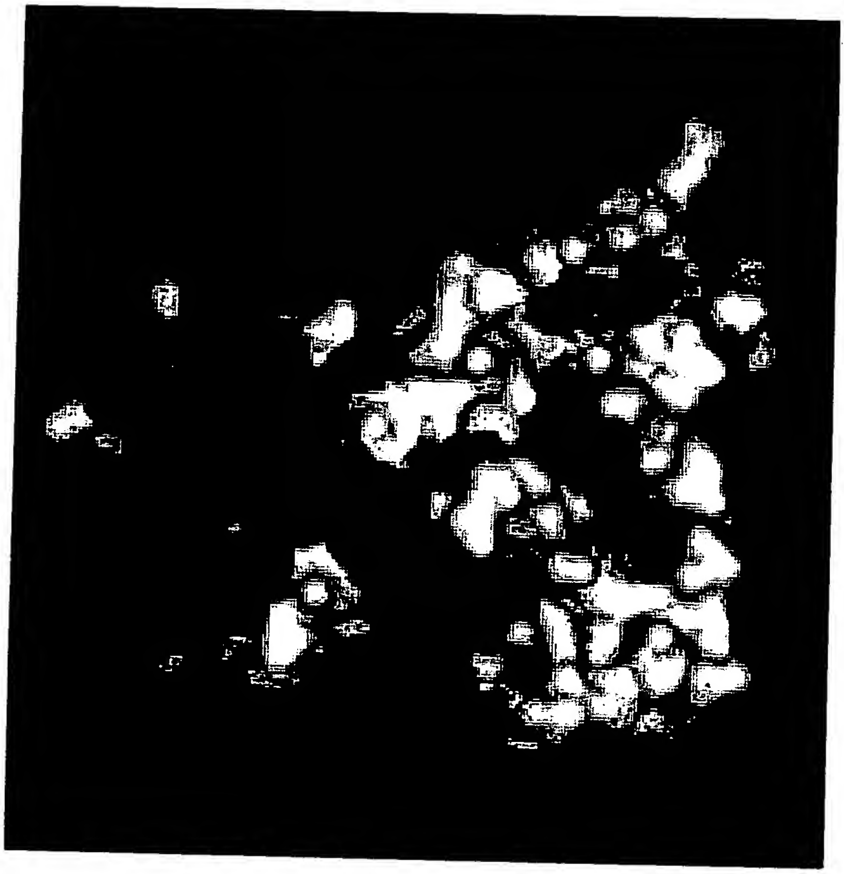
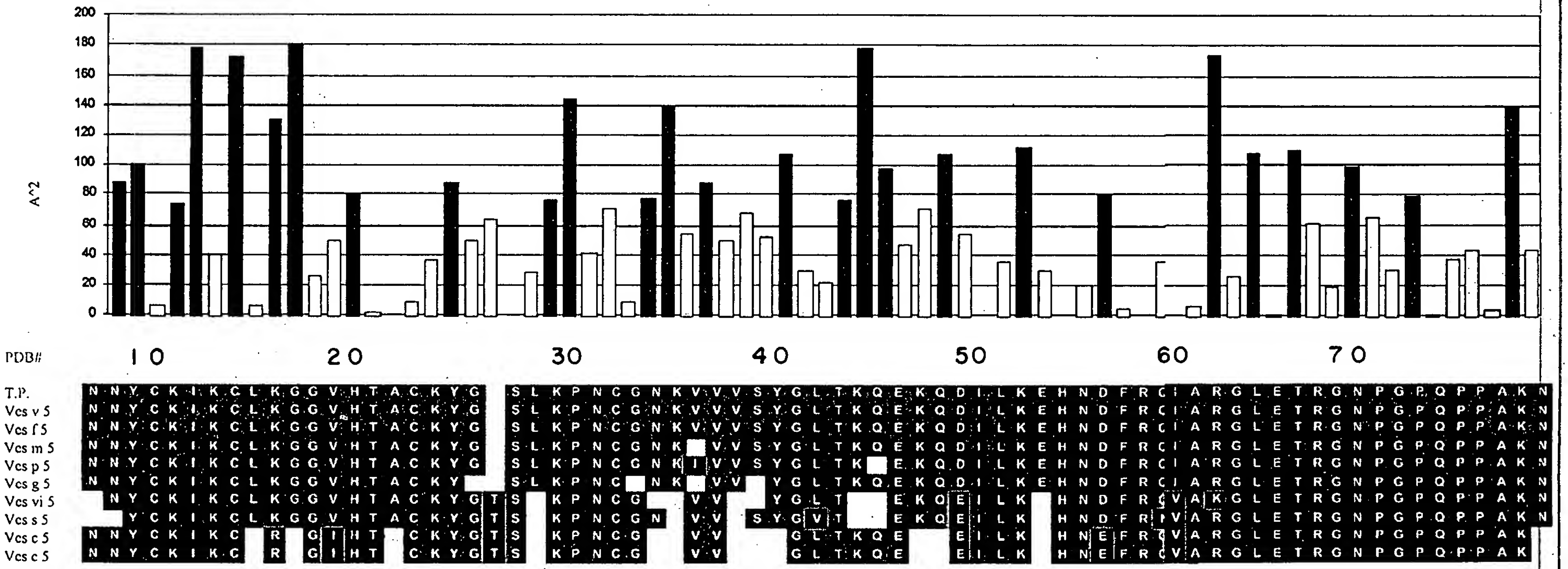


FIG. 10B


A^2
200
180
160
140
120
100
80
60
40
20
0
PDB#
10
20
30
40
50
60
70
T.P. N N Y C K I K C L K G G V H T A C K Y G   S L K P N C G N K V V V S Y G L T K Q E K Q D I L K E H N D F R C I A R G L E T R G N P G P Q P P A K N
Vcs v 5 N N Y C K I K C L K G G V H T A C K Y G   S L K P N C G N K V V V S Y G L T K Q E K Q D I L K E H N D F R C I A R G L E T R G N P G P Q P P A K N
Vcs f 5 N N Y C K I K C L K G G V H T A C K Y G   S L K P N C G N K V V V S Y G L T K Q E K Q D I L K E H N D F R C I A R G L E T R G N P G P Q P P A K N
Vcs m 5 N N Y C K I K C L K G G V H T A C K Y G   S L K P N C G N K   V V S Y G L T K Q E K Q D I L K E H N D F R C I A R G L E T R G N P G P Q P P A K N
Vcs p 5 N N Y C K I K C L K G G V H T A C K Y G   S L K P N C G N K I V V S Y G L T K   E K Q D I L K E H N D F R C I A R G L E T R G N P G P Q P P A K N
Vcs g 5 N N Y C K I K C L K G G V H T A C K Y   S L K P N C   N K   V V   Y G L T K Q E K Q D I L K E H N D F R C I A R G L E T R G N P G P Q P P A K N
Vcs vi 5   N Y C K I K C L K G G V H T A C K Y G T S   K P N C G   V V   Y G L T   E K Q E I L K   H N D F R C V A K G L E T R G N P G P Q P P A K N
Vcs s 5   Y C K I K C L K G G V H T A C K Y G T S   K P N C G N   V V   S Y G V T   E K Q E I L K   H N D F R I V A R G L E T R G N P G P Q P P A K N
Vcs c 5 N N Y C K I K C   R   G I H T   C K Y G T S   K P N C G   V V   G L T K Q E   E I L K   H N E F R C V A R G L E T R G N P G P Q P P A K
Vcs c 5 N N Y C K I K C   R   G I H T   C K Y G T S   K P N C G   V V   G L T K Q E   E I L K   H N E F R C V A R G L E T R G N P G P Q P P A K

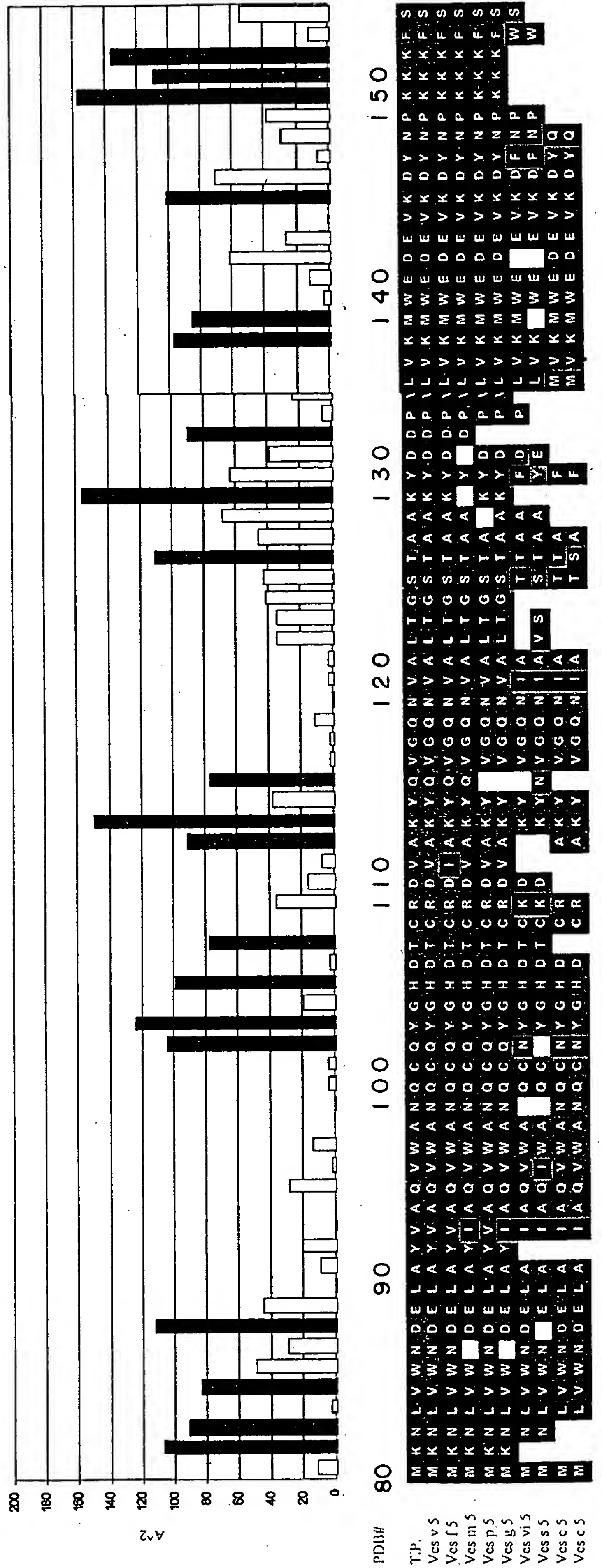

200
180
160
140
120
100
80
60
40
20
0
A^2
80
90
100
110
120
130
140
150
PDB#
T.P.
Vcs v 5
Vcs f 5
Vcs m 5
Vcs p 5
Vcs g 5
Vcs vi 5
Vcs s 5
Vcs c 5
Vcs c 5

FIG. 10C

FIG. 10D

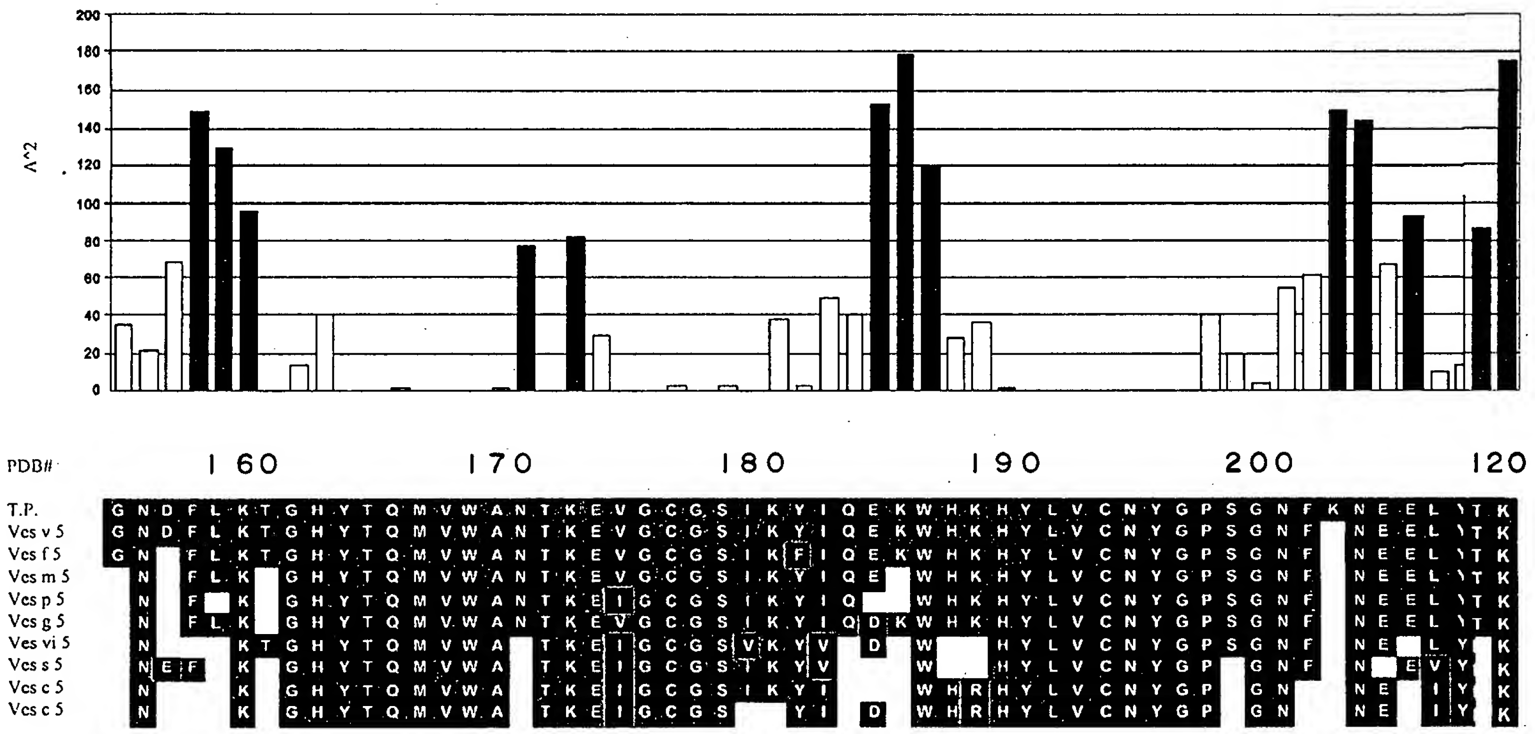

A^2
200
180
160
140
120
100
80
60
40
20
0
PDB#
160
170
180
190
200
120
T.P. G N D F L K T G H Y T Q M V W A N T K E V G C G S I K Y I Q E K W H K H Y L V C N Y G P S G N F K N E E L Y T K
Vcs v 5 G N D F L K T G H Y T Q M V W A N T K E V G C G S I K Y I Q E K W H K H Y L V C N Y G P S G N F N E E L Y T K
Vcs f 5 G N F L K T G H Y T Q M V W A N T K E V G C G S I K F I Q E K W H K H Y L V C N Y G P S G N F N E E L Y T K
Vcs m 5 N F L K G H Y T Q M V W A N T K E V G C G S I K Y I Q E W H K H Y L V C N Y G P S G N F N E E L Y T K
Vcs p 5 N F K G H Y T Q M V W A N T K E I G C G S I K Y I Q W H K H Y L V C N Y G P S G N F N E E L Y T K
Vcs g 5 N F L K G H Y T Q M V W A N T K E V G C G S I K Y I Q D K W H K H Y L V C N Y G P S G N F N E E L Y T K
Ves vi 5 N K T G H Y T Q M V W A T K E I G C G S V K Y V D W H Y L V C N Y G P S G N F N E L Y K
Vcs s 5 N E F K G H Y T Q M V W A T K E I G C G S T K Y V W H Y L V C N Y G P G N F N E V Y K
Vcs c 5 N K G H Y T Q M V W A T K E I G C G S I K Y I W H R H Y L V C N Y G P G N N E I Y K
Vcs c 5 N K G H Y T Q M V W A T K E I G C G S Y I D W H R H Y L V C N Y G P G N N E I Y K

FIG. 11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants.
Mutated nucleotides underlined.

```
Ves v 5 mutant 1 (K72A)
Ves v 5 sense       5'- A C C A C A G C C T C C A G C G A A G A A T A T G A A A A A T T T G G T A T G G A -3'
Ves v 5 non-sense   3'- T G G T G T C G G A G G T C G C T T C T T A T A C T T T T T A A A C C A T A C C T -5'
sense primer        5'-                     C C A G C G G C T A A T A T G A A A A A T                     -3'
non-sense primer    3'-         G T C G G A G G T C G C C G A T T A T A C                                 -5'

Ves v 5 mutant 2 (Y96A)
Ves v 5 sense       5'- G G C T A A T C A A T G T C A A T A T G G T C A C G A T A C T T G C A G G G A T G -3'
Ves v 5 non-sense   3'- C C G A T T A G T T A C A G T T A T A C C A G T G C T A T G A A C G T C C C T A C -5'
sense primer        5'-                     T G T C A A G C T G G T C A C G A T A C T                     -3'
non-sense primer    3'-         T T A G T T A C A G T T C G A C C A G T G                                 -5'
```

FIG. 12

Oligonucleotide primers for the site directed mutagenesis of Ves v 5.

all sense 1 : XhoI start, 38-mer:

```
          EcoRI
5'-C C G C T C G A G A A A A G A A A C A A T T A T T G T A A A A T A A A A T G
         L     E     K     R     N     N     Y     C     K     I     K
    KaX2  cleavage site              amino terminus of Ves v 5
```

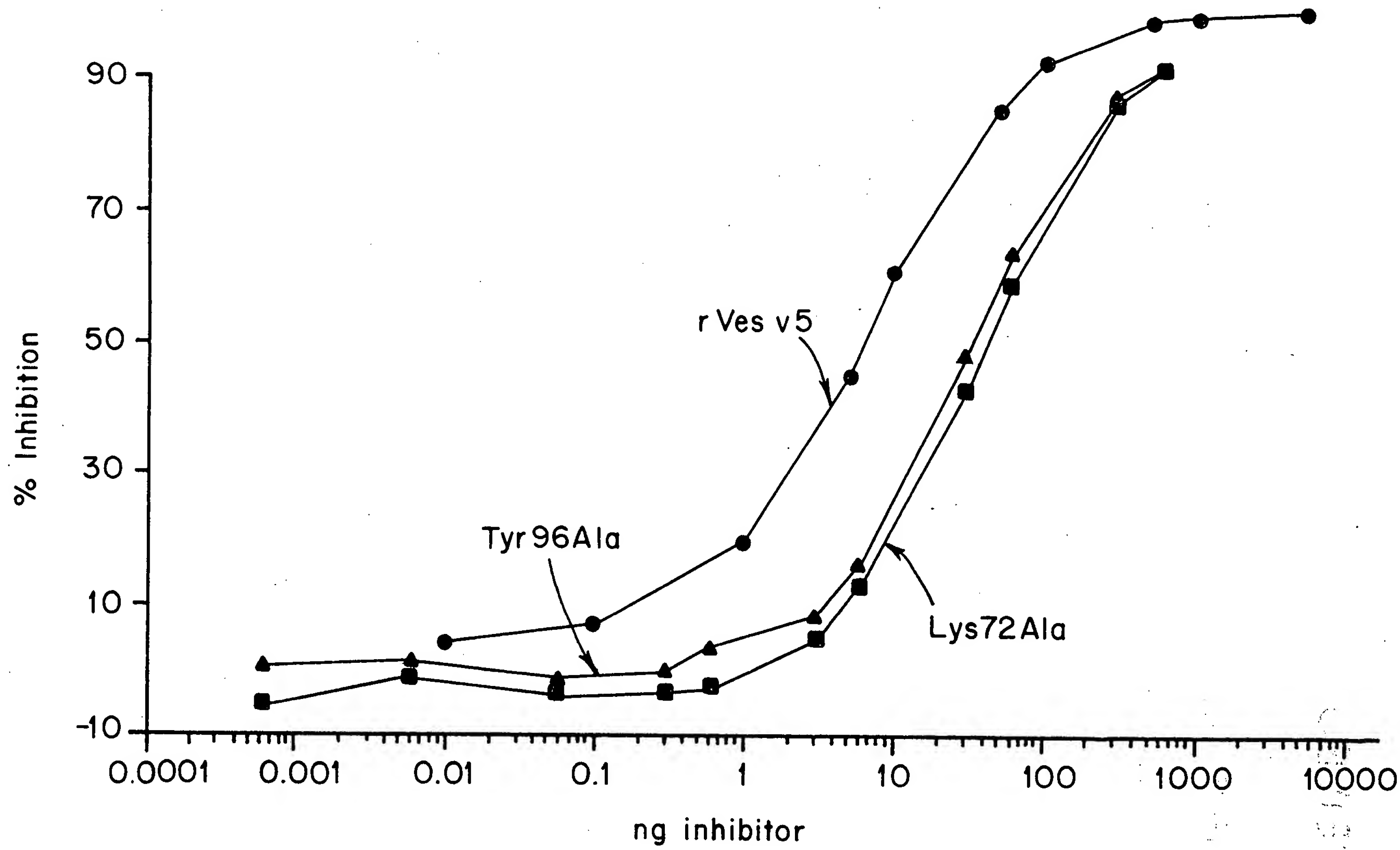
1	sense	1 : K72As	21-mer	5'- C C A G C G G C T A A T A T G A A A A A T
1	non-sense	2 : K72Aa	21-mer	5'- C A T A T T A G C C G C T G G A G G C T G
2	sense	3 : Y96As	21-mer	5'- T G T C A A G C T G G T C A C G A T A C T
2	non-sense	4 : Y96Aa	21-mer	5'- G T G A C C A G C T T G A C A T T G A T T
all	non-sense	7 : CT-pPICZaA,	21-mer	5'- A T T C A T C A G C T G C G A G A T A G G

DRAFTSMAN

FIG. 13

```
  1 AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA   60
  1  N  N  Y  C  K  I  K  C  L  K  G  G  V  H  T  A  C  K  Y  G   20

 61 AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG  120
 21  S  L  K  P  N  C  G  N  K  V  V  V  S  Y  G  L  T  K  Q  E   40

121 AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG  180
 41  K  Q  D  I  L  K  E  H  N  D  F  R  Q  K  I  A  R  G  L  E   60

                                           1 (K72A) (AAG- GCT)
181 ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGAAC  240
 61  T  R  G  N  P  G  P  Q  P  P  A  K  N  M  K  N  L  V  W  N   80

                                                    2 (Y96A) (TA -GC)
241 GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT  300
 81  D  E  L  A  Y  V  A  Q  V  W  A  N  Q  C  Q  Y  G  H  D  T  100

301 TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT  360
101  C  R  D  V  A  K  Y  Q  V  G  Q  N  V  A  L  T  G  S  T  A  120

361 GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGGGAAGATGAAGTGAAAGATTAT  420
121  A  K  Y  D  D  P  V  K  L  V  K  M  W  E  D  E  V  K  D  Y  140

421 AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG  480
141  N  P  K  K  K  F  S  G  N  D  F  L  K  T  G  H  Y  T  Q  M  160

481 GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG  540
161  V  W  A  N  T  K  E  V  G  C  G  S  I  K  Y  I  Q  E  K  W  180

541 CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT  600
181  H  K  H  Y  L  V  C  N  Y  G  P  S  G  N  F  K  N  E  E  L  200

601 TATCAAACAAAGTAA                                               612
201  Y  Q  T  K  stop                                             204
```

FIG. 14


90
70
50
30
10
-10
% Inhibition
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
ng inhibitor
r Ves v5
Tyr96Ala
Lys72Ala